

## *Helicobacter pylori*

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### ***Helicobacter pylori*-induced Apoptosis in Gastric Cancer Cell Lines**

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**Background/Aims:** *Helicobacter pylori* (*H. pylori*) is associated with active gastritis and peptic ulcer disease. Mechanism for *H. pylori*-induced gastric epithelial damage is still incompletely understood. However, the increase of apoptotic cells in *H. pylori*-infected mucosa suggested that apoptosis could be a major mechanism for cellular damage. As an effort to clarify the mechanism, we investigated whether *H. pylori* directly induce apoptosis in gastric cancer cells in vitro. **Methods:** Cultured *H. pylori* (ATCC 43504) were suspended as 10<sup>9</sup>mL. IL (interleukin)-8 was measured by enzyme linked immunosorbent assay. Cell survival was assessed by MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. Apoptosis was detected and confirmed by demonstration of DNA fragmentation and morphologic changes. **Results:** *H. pylori* induced IL-8 production as well as decrease of cell survival in gastric cancer cell lines in a time- and concentration-dependent way. Addition of *H. pylori* to gastric cancer cells induced apoptosis. Such induction was not organ specific. Heat or formalin treatment of *H. pylori* almost completely inhibited IL-8 production but only partially blocked apoptosis. *H. pylori*-induced apoptosis was potentiated by interferon- $\gamma$  pretreatment in HT-29 but not in AGS and KATO III. **Conclusions:** These results suggest that *H. pylori* affects on gastric epithelial cell growth by direct induction of apoptosis. (Kor J Gastroenterol 1999;34:21 - 34)

**Key Words:** *Helicobacter pylori*, Apoptosis, Interleukin-8

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*Helicobacter pylori*

- 14 *H. pylori*  
*H. pylori*가  
56 *H. pylori*가  
가  
78 *H. pylori*  
inducible nitric oxide synthase  
(iNOS) mRNA가, 4913 *H. pylori*  
*pylori*  
chemokine가  
가  
*H. pylori*가 urease  
가, 1820 *H. pylori*  
cell)가 *H. pylori* (apoptotic가  
.2421,22  
(apoptosis, programmed cell death)  
genomic DNA가 endonuclease  
180 oligomer  
가, chromatin  
body) (apoptotic  
.2324 ATP  
, ,  
(ligand) , DNA  
.2527  
molding  
.2829
- H. pylori*  
.243032  
. , *H. pylori*가 urease가  
*H. pylori*가 . *H.*  
가  
가  
*H. pylori*  
22 *H. pylori*  
*H. pylori*  
가  
*H. pylori*  
.  
1.  
KATO III (MTB-38, European  
Collection of Animal Cell Cultures, Salisbury, Wilts)  
AGS (ATCC CRL 1739, Rockville, MD) ,  
HT-29 (ATCC HTB38), DLD-1 (AT-  
CC CCL-221) HCT-15 (ATCC CCL-225) 40 µg/  
mL gentamicin 10% (fetal  
calf serum:FCS) Dulbecco's modified Ea-  
gle Medium (DMEM) 5% CO<sub>2</sub> 37  
*H. pylori* 가  
가  
2. *H. pylori*  
Cytotoxin/*cagA* ATCC 43504  
(NCTC 11637) . *H. pylori*  
37  
3-5  
가 10  
*H. pylori*  
10 mM phosphate buffered saline (PBS; pH  
7.4) McFarland 3 (1 × 10<sup>9</sup> /mL)

10% FCS 가  
70

3.

MTT assay .33  
96-well microtiter plate (Costar, Cambridge,  
MA) (104/well) 가  
0, 2  
20, 200, 600, 1,200 가  
*H. pylori* 가

well 50  $\mu$ L 2  $\mu$ g/mL MTT [3-(4,5-dimethyl-  
thiazol-2-yl)-2,5-diphenyltetrazolium bromide]  
(Sigma) 가 4 37  
formazan

well 50  $\mu$ L  
dimethyl sulfoxide (DMSO) 가 10  
570 nm optical density (OD)  
Triton X-100 가  
well OD total OD

$$\% \text{Survival} = \frac{\text{sample OD} - \text{total OD}}{\text{spontaneous OD} - \text{total OD}} \times 100$$

#### 4. DNA fragmentation

10 mM Tris (pH 7.6), 10 mM EDTA, 50  
mM NaCl, 0.2% SDS 200  $\mu$ g/mL proteinase K  
42  
4 , 16,000 x g 20  
phenol-chloroform-  
isoamyl alcohol (25:24:1, Sigma) chloroform  
DNA DNA  
0.3 M sodium acetate ethanol  
0.2 U RNase A (Sigma) 가 10 mM Tris  
1 mM EDTA (pH 8.5) 30

RNA 1.2% agarose gel  
ethidium bromide

.3435

#### 5. IL (interleukin)- 8

IL-8 (ELISA)

96 well microtiter plate  
borate buffered saline (1.03%  $\text{H}_2\text{PO}_4$  0.73%  
NaCl; BBS) 200 goat anti-human IL-8  
polyclonal immunoglobulin G (R&D Systems, Min-  
neapolis, MN) well 100  $\mu$ L  
10 mM PBS (pH 7.4)/0.05% (v/v) Tween 20  
3 . 0.5% BSA/PBS

(recombinant human IL-8, R&D Systems) 가  
2 . PBS/Tween 3  
0.5% BSA/PBS 400 rabbit  
anti-human IL-8 polyclonal antibody (Endogen, Cam-  
bridge, MA) well 100  $\mu$ L 가 2  
PBS/Tween 3  
alkaline phosphatase 가 conjugate goat  
anti-rabbit IgG antibody (Jackson Laboratories, Avon-  
dale, PA) 1,000 100  $\mu$ L 가 30  
PBS/Tween 3 , Tris/NaCl

3 1 mg/mL disodium p-nitro-  
phenyl phosphate (Life Technologies, Gaithersburg,  
MD) 가 15 [3% 2-  
propanol, 1 mM iodonitrotetrazolium violet, 75  $\mu$ g/  
mL, alcohol dehydrogenase 50  $\mu$ g/mL diaphorase;  
Life Technologies] 가 10 ELISA  
492 nm OD

#### 6. (Electrophoretic mobility shift assay)

Tris-buffered saline (TBS)  
[10 mM HEPES, 10 mM KCl, 0.2  
mM EDTA, 1 mM DTT 가 protease inhi-  
bitor (1 mM phenylmethylsulfonyl fluoride (PMSF),  
5  $\mu$ g/mL aprotinin, 5  $\mu$ g/mL antipain, 100  $\mu$ M ben

zanidine, 5 µg/mL leupeptin, 5 µg/mL soybean trypsin-chymotrypsin inhibitor, pH 7.9)

15 0.625%가  
Nonidet P-40 가 . Tube 10 vortex  
12,500 x g 5  
[20 mM HEPES (4-[2-hydroxy-ethyl]-1-piperazineethanesulfonic acid), 400 mM NaCl, 1 mM EGTA (ethylene glycol-bis-{ -aminoethyl ether}-N,N,N',N'-tetraacetic acid), 1 mM DTT 7 가 protease inhibitors, pH 7.9)]

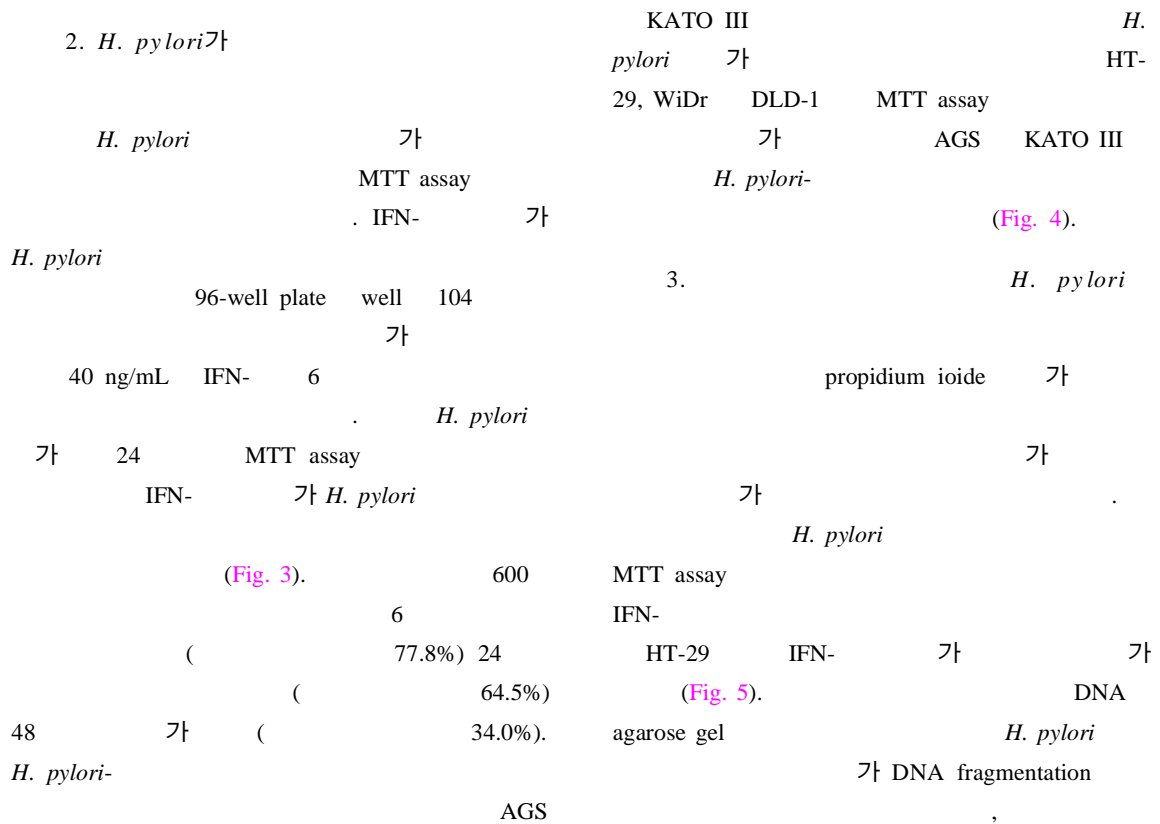
15 12,500 x g 20  
-70  
NF-κB  
5'-TAA CAA ACA GGG ATT TCA CCT ACA T-3'  
DNA

[ - P32]ATP (Amersham) T4 polynucleotide kinase (New England Biolabs, Beverly, MA)  
(label) 3-6 µg, 20,000 cpm  
2 µg poly dI/dC 10 mM Tris, 50 mM NaCl, 2 mM EDTA, 1 mM DTT 5% v/v glycerol (pH 7.5)  
60 4% polyacrylamide gel gel autoradiography

1. *H. pylori* IL-8  
AGS KATO III *H.*  
*pylori* IL-8  
가 (Fig. 1), electrophoretic mobility shift assay AGS *H. pylori*  
NF-κB binding activity  
가 (Fig. 2).

**Fig. 1.** *H. pylori* induced IL-8 protein production of gastric cancer cells. IL-8 productions by AGS and KATO III cells, measured by ELISA, increased dose-dependently.

**Fig. 2.** *H. pylori* dose-dependently activated NF-κB in AGS cells. NF-κB activation was measured by electrophoretic mobility shift assay at *H. pylori* to AGS cell ratio of 500 (lane 1), 100 (lane 2), 50 (lane 3), 10 (lane 4), 0 (lane 5) and HeLa cells as a positive control (lane 6).



**Fig. 3.** *H. pylori* induced decrease of cell survival of AGS (A) and KATO III (B). Indicated bacteria to cell ratio of *H. pylori* were added to gastric cancer cells and cocultured for 24 hours, then cell survival was measured by MTT assay.

AGS HT-29 (Fig. 7),  
 "ladder pattern" (Fig. 6). (transmission electron microscopy; TEM)  
 AGS *H. pylori* 가 ,  
 (scanning electron microscopy; SEM) (vacuole)  
 , *H. pylori* (Fig. 8) *H. pylori*가  
 coccoid form AGS .

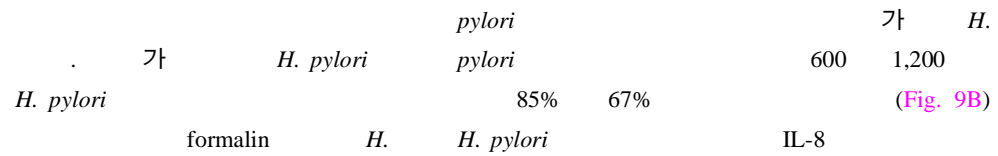
**Fig. 4.** *H. pylori*-induced decrease of cell survival was not organ-specific. Indicated bacteria to cell ratio of *H. pylori* were added to gastric cancer cell lines as well as various colon cancer cell lines and cocultured for 24 hours, then cell survival was measured by MTT assay.

**Fig. 5.** IFN- pretreatment potentiated *H. pylori*-induced apoptosis in colon cancer cell line HT-29. Indicated bacteria to cell ratio of *H. pylori* were added and cocultured with control (upper row) and IFN- pretreated (lower row) HT-29 cells for 16 hours. Propidium iodide was added to suspension of harvested cells. Using flow cytometer, cell size was measured by forward scatter and cytoplasmic membrane permeability was measured by red fluorescence.

**Fig. 6.** *H. pylori* induced DNA fragmentation of colon cancer cell line HT-29. DNA was extracted from control and cells cocultured for 48 hours with indicated bacteria to cell ratio of *H. pylori* was electrophresed on 1.2% agarose gel.

**Fig. 7.** *H. pylori* tightly adhered to gastric cancer cell line AGS. AGS cells were incubated with *H. pylori* (bacteria to cell ratio of 600) for 3 hours. Harvested cell pellets were fixed with 2% glutaraldehyde in 0.1 M cacodylated buffer, post-fixed in 1% osmium tetroxide, dehydrated in ethanol gradient and coated with gold (Hitachi S-800, ×6,000).

		0.5% formaldehyde		가	4		
4. <i>H. pylori</i>		PBS			.	가	
		formalin	<i>H. pylori</i>		IL-8		
<i>H. pylori</i>		가	formalin	<i>H. pylori</i>			
		PBS	500	70%	95%		
<i>H.pylori</i> (5 × 10 <sup>8</sup> mL)	90	15	가	(Fig. 9A)	IL-8		

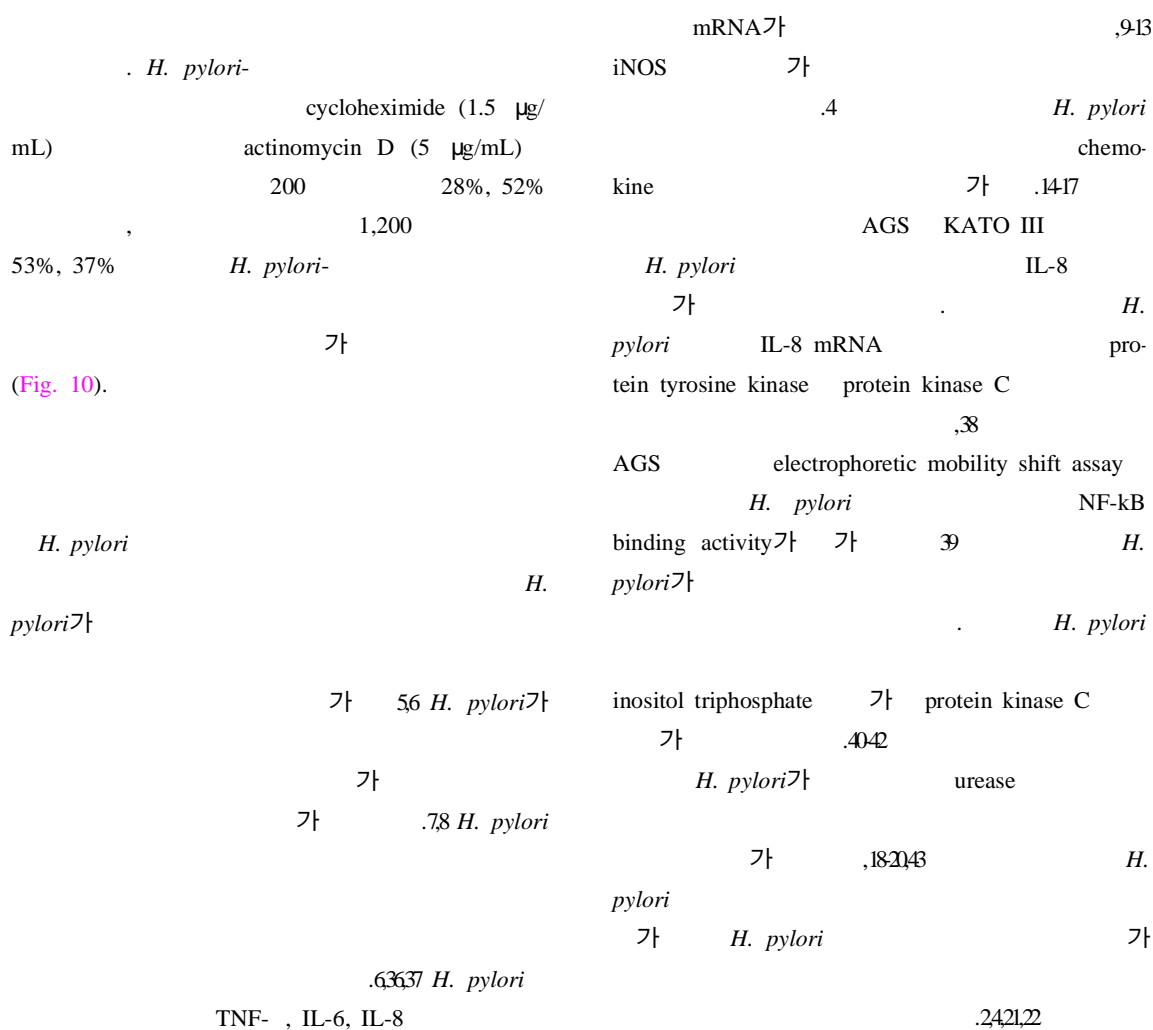


**Fig. 8.** *H. pylori* induced cytoplasmic vacuolization and chromatin condensation of gastric cancer cell line, AGS. AGS cells were incubated with *H. pylori* (bacteria to cell ratio of 600) for 48 hours. Harvested cell pellets were fixed with 2% glutaraldehyde in 0.1 M cacodylated buffer, post-fixed in 1% osmium tetroxide, dehydrated in ethanol gradient and embedded in Epon. Then 0.5 m thin cut section was prepared and double stained with uranyl acetate and lead citrate (Phillips CM 10,  $\times$  6,000).

**Fig. 9.** Effect of heat and formalin treatment of *H. pylori* on IL-8 production (A) and cell survival (B) of AGS cells. (A) Control as well as heat and formalin treated *H. pylori* (bacteria to cell ration of 500) were cocultured with AGS for 16 hours and IL-8 production was measured by ELISA. Values represent percent production of IL-8 compared to control *H. pylori*. (B) Indicated bacteria to cell ratio of *H. pylori* were added to AGS cells and cocultured for 24 hours, then cell survival was measured by MTT assay. Values represent percent cell survival of AGS cells compared to control *H. pylori*.



**Fig. 10.** Cycloheximide and actinomycin D inhibited *H. pylori*-induced decrease of gastric cancer cell survival. AGS cells were pretreated with 1.5  $\mu$ g/ml of cycloheximide or 5  $\mu$ g/ml of actinomycin D before the addition of indicated bacteria to cell ratio of *H. pylori*, and then cocultured for 24 hours. Cell survival was measured by MTT assay.



<i>H. pylori</i>	가	tern"	
	MTT assay		
	,		<i>H. pylori</i> -
6	24		
	48	가	
		phosphatidylserine	
<i>H. pylori</i>		Annexin V assay	<i>H.</i>
		<i>pylori</i> 가	
IL-8	가	.4546	
	가	<i>H. pylori</i>	
KATO III	<i>H. pylori</i>	AGS	cyto-
	<i>H. pylori</i> -	toxin associated gene (CagA)	vacuolating cyto-
		toxin (VacA)	<i>H. pylori</i> 가
		CagA	
	가	VacA <i>H. pylori</i> 가	
	34 IFN-	가 <i>H.</i>	
<i>pylori</i>		가	.947 CagA 가
	<i>H. pylori</i>		.1417 CagA
IFN-		VacA <i>H. pylori</i>	
HT-29	IFN-	가	
가	IFN-		(surface factor)
		가	<i>H. pylori</i> ,
MHC class II	가	sonificate4849	
44	, IFN-		40 <i>H.</i>
MHC class II	가	<i>pylori</i> 가	
IFN-	가		<i>H. pylori</i>
	가		
DNA fragmentation		CagA ,14 flagella,48 urease,50 lipo-	
		polysaccharide (LPS),51 cytotoxin	
		<i>H. pylori</i>	
DNA fragmentation		LPS, (water extrac-	
		table surface protein) urease	
	DNA agarose	1651	
gel	<i>H. pylori</i>	, 가	formalin <i>H.</i>
	가 DNA fragmentation	<i>pylori</i>	IL-8
	AGS	<i>H. pylori</i>	70% 95%
	HT-29	"ladder pat-	IL-8

electrophoretic mobility shift assay 가 .  
: AGS KATO III *H. pylori*  
IL-8  
가 . *H. pylori*  
. *H. pylori*-  
. 가 formalin  
IL-8  
*H. pylori*  
*H. pylori* 70% 95%  
가 *H. pylori*  
*H. pylori*  
, formalin  
가 . *H. pylori*-  
cyclohexi-  
mide actinomycin D  
. : *H. pylori*가  
.  
*H. pylori*  
coccoid form AGS  
*H. pylori*가  
가 .  
가 .

: *Helicobacter pylori* (*H. pylori*)

*H. pylori*

. *H. pylori*

. *H. pylori*가

. : *H. pylori* cytotoxin/cagA  
ATCC 43504 (NCTC 11637)

. MTT assay ,  
agarose gel DNA fragmentation

. IL-8 ELISA NF-kB

: *Helicobacter pylori*, (apoptosis),  
Interleukin-8

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Fas (apoptosis)  
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